Spatially-distributed viral evolutionary analysis of Influenza A Virus (IAV)

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Background. Influenza A is a virus within the *Orthomyxoviridae* family causing an acute contagious viral respiratory infection and circulating among people worldwide. In humans, influenza A viruses cause yearly outbreaks with high morbidity and excess fatality rates. Influenza A natural hosts are ducks, where it occurs as a benign disease. It can infect and adapt to various hosts using error-prone replication that allow to undergo rapid genetic change by recombining with other influenza viruses (WOAH, 2021). The adaptation of the virus depends on the receptor-binding specificity and stability of hemagglutinin (HA) protein, neuraminidase (NA) receptor-destroying activities, polymerase (PA) efficiency *etc.* People usually infected by human-specific subtypes, birds — by the avian ones because of different combination and location of receptors in respiratory system. Pigs and ferrets can be infected by both viral subtypes. Thus, these species are known as potential sources of new variants of IV with ability to cross species transmission. The aim of the study was testing if the positive selection drives the evolution of HA\NA\PA by favoring mutations that enhance viral fitness, such as by increased binding affinity to host receptors or evasion of host immune responses. We also tested if the swine are the hosts in which virus selection is intensified. **Methods.** For testing the positive selection hypothesis, we used unique NCBI sequences of HA/NA/PA from New York (42 seqs), Egypt (616, 167, 134 seqs) and Bangladesh (637, 452, 393 seqs). Sequences were aligned in MEGA 11, manually QC-checked and cleaned in AliView. The evolutionary models were checked using BUSTED algorithm with threshold equal 10. For testing the selection in swine hosts, we used a dataset of PA sequences of 21 swine samples and subsets of the Egypt/Bangladesh/NY datasets. Swine samples were marked as a test group and analyzed by RELAX algorithm. Both algorithms were tested on DataMonkey (datamonkey.org) web page. **Results and conclusions.** As expected, the chosen proteins are under positive selection. We found the evidence of virus positive selection in the PA samples from New York (27 sites), HA and NA samples from Egypt (17 and 16 sites, respectively). The most often mutations for HA were found in mature peptide HA1 domain, for NA — in sialidase β-propeller 6 structural motif, and PA — in Arch domain. No evidence of positive selection for all 3 proteins for the samples from Bangladesh, that can be a signal of slow evolutionary activity at this country. The testing of evolution in swine hosts showed that selection is significantly relaxed in the test group. Which means that genetic drift, but not a selection is a main force in the test group. The result might be different in datasets with more swine samples.

K e y w o r d s: Influenza A, virus positive selection, virus evolution.